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REVIEW OF REACTIONS OF BIOTOXINS IN WATER
(CBIAC TASK 152)

Final Report

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A review of the literature, both open and classified, was conducted on the hydrolytic and photolytic reactions and reactions with common disinfectants of U.S. and suspected threat biotoxins in dilute aqueous solution. References were reviewed covering Botulinum toxin A, Tetanus toxin, Diphteria toxin, Ricin, Palytoxin, Tetrodotoxin, Saxitoxin, Conotoxin, Microcystin, and Anatoxin A. Information obtained includes estimates of stability under various pH and temperature conditions.

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FOREWORD

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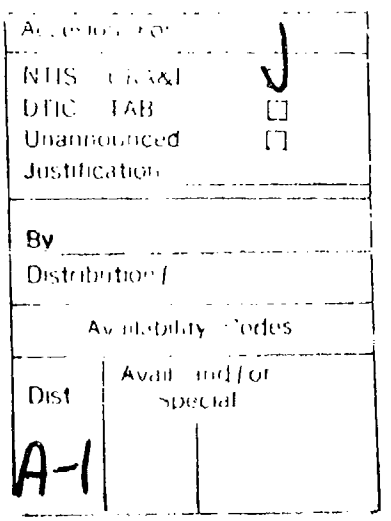

INTRODUCTION

In a battlefield contaminated with biotoxins used in warfare, water supplies may be contaminated intentionally or incidentally. Part of the mission of of the U.S. Army Biomedical Research and Development Laboratory (USABRDL) concerns evaluating the potential for adverse human health effects from consumption of contaminated water. It is essential that the fates of biotoxins in water subjected to Army field water treatment be learned.

This report describes a literature review conducted to obtain a data base of hydrolysis and photochemical reactions of biotoxins and reactions of biotoxins with common disinfectants.

SCOPE OF WORK

The review covered both open and classified literature. The work was directed specifically to obtaining information on the four protein neurotoxins and six nonprotein neurotoxins listed below.

Protein Neurotoxins	Nonprotein Neurotoxins
Botulinum toxin A	Palytoxin
Tetanus toxin	Tetrodotoxin
Diphtheria toxin	Saxitoxin
Ricin	Conotoxin
	Microcystin
	Anatoxin A

Data sought on hydrolysis reactions included solubilities, reaction rates, and reaction products of biotoxins in water, with particular emphasis on concentrations below 1 g/L. The effects of temperature, pH, ionic strength, and general or specific catalysis were considered

of importance, as well as, discussions of reaction mechanisms when such information might contribute to an understanding of the fates of biotoxins in natural waters.

Data sought on photochemical reactions included reaction rates, quantum yields, and photochemical degradation products of biotoxins in dilute aqueous solution, particularly when such data might be relevant to the fate of biotoxins in natural waters.

Data sought on reactions with disinfectants included reaction rates and reaction products, particularly data from reactions with dilute aqueous solutions of hypochlorite or iodine. Decontamination data was of interest if no other data were available.

DESCRIPTION OF INFORMATION SEARCH

Computer-assisted on-line searches of the following data bases were conducted.

- Chemical Abstract Service (CAS)
- National Technical Information Service (NTIS)
- Defense Technical Information Center (DTIC)
- Chemical/Biological Information Analysis Center (CBIAC)
- BIOSIS
- MEDLINE

The key limiters used for the searches were the names and CAS registry numbers of each of the ten toxins, and the following terms:

hydrolysis	photochemical	reaction rate	breakdown
water	solubility	chlorine	fate
destruction	aqueous	quantum yield	iodine
reaction	disinfect	aquatic	chemical
hypochlorite	degradation	destroy	reaction

In addition to the computer-assisted searches, a manual search of Chemical Abstracts for the years 1946 to 1967 was conducted.

RELEVANT INFORMATION RETRIEVED

The computerized searches yielded the following numbers of references.

- CAS - 356
- MEDLINE - 474
- BIOSIS - 513
- NTIS - 23
- DTIC/CBIAC - 271

Despite the large numbers of "hits" obtained, very little relevant information was found. Most of the references were devoted to isolation, synthesis, toxicology, or biological studies. The abstracts of all of the references were reviewed. Hard copies of the complete original references were obtained for about 40 of the more relevent citations and reviewed in detail.

The most useful reference was a review of chemical, biological, and toxicological properties of selected toxins and venoms conducted in 1983 by Thomas Facklam, of Battelle's Tactical Technology Center, for the U.S. Army Chemical Systems Laboratory.[8] Facklam's review covered five of the ten toxins of interest to the current program. The information provided by references covered by Facklam's review and other references is presented in the following sections.

Botulinum Tox'n A

Botulinum toxin can be isolated in crystalline or lyophilyzed form, can be destroyed or inactivated by alkali, chlorine developer, or iodine.[7] Water infected with botulinum toxin was decontaminated by chlorination, boiling, sterilization, or charcoal treatment.[17] Vigorous agitation of a dry blend of the toxin in air at 24°C causes a loss of activity.[22] Silverman et al. in studying the environmental persistence of botulinum neurotoxin A on surfaces, found that the

toxin is stabile enough to require detoxification.[24] These workers also found that the effectiveness of the various detoxifying agents tested was dependent upon temperature, pH, and purity of the toxin.

Tetanus Toxin

Tetanus toxin was found by Lahiri and Dutta to be denatured rapidly under acidic conditions; purification was conducted in the cold to avoid degradation.[16] In work conducted by Helting and Zwisler on the enzymatic degradation of tetanus toxin, the toxin was hydrolyzed with papain at pH 6.5 at 55°C but was stable below 40°C.[13] Wright et al. in work on the development and evaluation of a purified tetanus toxin, studied the stability of the toxin.[27]

Diphtheria Toxin

Blewitt et al. reported that diphtheria toxin switches from a hydrophilic conformation to a hydrophobic conformation at pH 4 to 5.[4] Ramsay et al. reported that the toxin is most stable at pH 7 to 8 and unfolds at high and low pHs.[21]

Ricin

Ricin is a glycoprotein having a molecular weight of 62,000 and consisting of two different polypeptide chains joined by a disulfide bond; the disulfide bond can be broken by incubating the toxin at 37°C for 3 hours in pH 8.5 Tris buffer containing 2 percent 2–mercaptoethanol.[9] Ricin was reported by Bushueva and Tonevitsky to be stable over the range of pH 3 through 8.[6] Taira et al. reported that intact ricin D is stable at pH 1 through 11 for 24 hours at 25°C and stable at 50°C for 1 hour at pH 7.8.[26]

Ricin is not hydrolyzed by trypsin or pepsin, but is partially hydrolyzed when incubated with an alkaline protease, nigrase, at 30°C and pH 8.0 for 16 hours; it is hydrolyzed by trypsin only in the presense of guanidine hydrochloride.[10, 29] Yamasaki et al. reported that ricin is denatured and insolubilized by freeze-thawing.[28]

Palytoxin

Palytoxin is a generic term for water-soluble toxins from a genus of soft corals. The toxins are moderately stable and have molecular weights of approximately 3,000. Moore and Scheuer have reported that the half-lives of palytoxin in 0.05 N HCl and 0.05 N NaOH are 85 and 55 minutes, respectively.[18]

Tetrodotoxin

Studies on tetrodotoxin have been reviewed by Goto et al. and Kao.[12, 14] The toxin is extracted by boiling water to prevent autolysis and is soluble in slightly acidic water. Mosher et al. have reported that tetrodotoxin is rapidly converted to the nontoxic tetrodonic acid if the acid is very strong.[19] The toxin is weakly basic, and, although it is insoluble in water under basic conditions, it is rapidly destroyed under such conditions.

Saxitoxin

The review by Kao indicates that saxitoxin is a basic substance that is very soluble in water as the dihydrochloride.[14] Burke et al. have reported that saxitoxin is water soluble and acid stable, and survives boiling with 0.15 M trichloroacetic acid for 30 minutes.[5] However, there is an appreciable loss in activity when the toxin is boiled in 0.03 N HCl for 30 minutes. The toxin decomposes rapidly in basic solution. Ghazarossian et al. have reported that saxitoxin is

hydrolyzed to decarbamoylsaxitoxin in 7.5 N HCl at 100°C for 3 to 8 hours without loss of activity.[11] Koehn et al. achieved the conversion by heating the toxin in 7.5 N HCl at 115°C under nitrogen for 90 minutes and reported a 40 percent loss in activity.[15] Rubinson has reported that the acid hydrolysis product is at least 100-fold less toxic than saxitoxin.[23]

Conotoxin

No information was found on the stability of contoxin.

Microcystin

Microcystin appears to be reasonably stable in water in that it leaches from cells into water over a 40-day period at 17°C.[2] A review by Bishop et al. indicates that there have been conflicting reports on the stability of microcystin ranging from unstable to heat when wet to stable when autoclaved in neutral solution.[3] Rabin and Darbre reported that there is no change in the toxicity of microcystin when it is heated for 1 hour at 100°C at pH 1 through 7.[20] Amann and Juettner have reported that microcystin is readily deactivated even when frozen.[1]

Anatoxin A

Smith and Lewis have reported that Anatoxin A in water under ambient conditions is converted to the nontoxic anhydro derivative in several days.[25]

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